"On the Toxic Properties of the Saliva of Certain 'Non-poisonous' Colubrines." By A. Alcock, M.B., LL.D., F.R.S., Professor of Zoology, and Leonard Rogers, M.D., B.S. (Lond.), M.R.C.P., F.R.C.S., Officiating Professor of Pathology in the Medical College of Bengal. Received April 28,—Read June 12, 1902.

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I. General Statement and Conclusions.

Although numerous elaborate experiments, directed chiefly towards practical ends, have been made to determine the physiological effects of the parotid secretion of those Colubrine snakes whose bite is fatal to man, yet very little seems to have been done, by studying the effects of the saliva of the non-poisonous Colubrines, to assist us in forming some opinion as to how, on the theory of gradual modification by means of natural selection, the efficient lethal mechanism of the poisonous Colubrines may be supposed to have originated and become gradually perfected in all its parts.

On comparing two strong, active reptiles like the common Cobra (*Naia tripudians*) and the common Rat-snake (*Zamenis mucosus*) of this country, both of which seem to lead—and with identical success—lives that are essentially similar, two perplexing questions occur. The first question is—what is the manifest advantage to the Cobra, over the Rat-snake, of its venom? The second is, admitting that there must be some advantage—is it conceivable that it can be founded on any fundamental and unbridged difference in the nature of the saliva of the two species?

A few experiments that we have made seem to point to the conclusion that the difference is not a radical one but is only one of degree, and that the parotid secretion of some of the "harmless" Colubrines is to a certain extent poisonous when injected subcutaneously.

That the poison-gland of the venomous snakes is merely a modified

parotid gland is a generally accepted fact. That various gradations exist between an ordinary serpent's tooth and the "perforated" tooth of the venomous snakes is equally well known. If then we can show that the parotid secretion of the "harmless" Colubrines is in any degree poisonous, and if, moreover, in a series of such harmless Colubrines we can find degrees of virulence, we shall, we think, have done something towards placing the venom apparatus of the Thanatophidia at one extreme of a natural series in harmony with other facts of evolution. At one end of this series we shall have a snake like the Rat-snake (*Zamenis mucosus*) with a parotid secretion that is small in amount and only slightly toxic, and with no special means of injecting its secretion; at the other end of the series we shall have the Cobra, whose abundant parotid secretion is of lethal virulence and can be powerfully injected by a specialised fang.

We admit that certain of the links in this series have already been discovered and established, but we do not think that their full evolutional value has yet been assigned to them.

For instance, Mr. Boulenger, in his 'Catalogue of Snakes,' says of the three Colubrine sub-families *Homalopsinæ*, *Dipsadomorphinæ*, and *Elachistodontidæ*, that constitute his section of Opisthoglypha, "most, if not all, of the snakes of this division are poisonous to a slight degree, paralysing their prey before deglutition."

Again, Mr. G. S. West,* though he apparently speaks with some reserve as to the toxicity of the Opisthoglypha in general, accepts the fact that "the bite of *Dryophis* and other Opisthoglyphous snakes has been proved by several observers to be fatal to small animals."

MM. Phisalix and Bertrand,† experimenting with two European species of *Tropidonotus*, a genus of Aglyphous or "harmless" Colubrines, discovered that the secretion of the salivary glands was fatal to guinea-pigs.

Earlier still, Mr. J. J. Quelch‡ recorded of two American Colubrines —one an Opisthoglyphous or "suspicious" species (*Erythrolamprus æsculapii*), the other an Aglyphous or "harmless" species (*Xenodon severus*)—that their bite could produce enduring and painful inflammation in man.

In seeking for ourselves some independent evidence for the popular belief that "all snakes are poison," we began with the Opisthoglyphous species *Cerberus rhynchops*, *Dipsas Forstenii*, *Dryophis mycterizans*, and *Chrysopelea ornata*, all of which have parotid glands of some size and have some of the posterior maxillary teeth enlarged and grooved.

We used white mice and white rats, and we injected subcutaneously the liquid from the (perfectly fresh) crushed parotid gland, and the

* 'Proceedings of the Zoological Society for 1895,' p. 813.

† 'Comptes Rendus,' vol. 118, 1894, pp. 76-79.

‡ 'Zoologist,' 1893, pp. 30, 31.

glycerine extract—both diluted and undiluted—of the gland. But as in a series of control experiments with the secretion of the Harderian gland, with the fresh serum, and with pure glycerine, we found that the subcutaneous injection of pure glycerine is sometimes fatal to rats and mice, we have altogether eliminated the results of experiments in which glycerine extract, diluted or undiluted, was used. Our recorded experiments, therefore, refer exclusively to the effects of the liquid extracted by water or normal salt solution from the parotid gland quickly removed from the still-quivering head of the decapitated snakes.

The only specimen of *Chrysopelea ornata* that we could obtain was a very young one, from which, as we expected, we got no results. But in the case of *Cerberus rhynchops*, *Dipsas Forstenii*, and *Dryophis mycterizans* we confirmed the statements and opinions of other authors as to the venomous character—so far as small mammals are concerned—of these Opisthoglyphous *Colubridæ*.

We next tried the effect of the parotid secretion of *Tropidonotus piscator* and *Zamenis mucosus*, both of which belong to Boulenger's section of *Aglypha*, or harmless Colubrines, none of whose teeth are grooved.

As before, we used white mice and white rats, and controlled our experiments so as to eliminate the influence of blood-serum and of glycerine; and we found that, so far as these small mammals are concerned, the parotid secretion of these two "harmless" Colubrines is decidedly toxic.

Nor, even in our small series of experiments, did we fail to find some evidence of that graduated variability which any one who approaches this question from the evolution side looks for. When all allowances are made, the parotid extract of *Zamenis* appears to be considerably more potent than that of the other Aglyphous Colubrine *Tropidonotus*; and, among the Opisthoglyphous snakes, *Dipsas* seems to be decidedly more virulent than *Cerberus*, and *Cerberus* slightly more venomous than *Dryophis*. It is further worthy of notice that the poison of the two Aglyphous snakes (*Zamenis* and *Tropidonotus*) appears to act more on the nervous system, causing general convulsions, while that of the Opisthoglyphous snakes seems rather to affect the respiratory centre and to occasion respiratory convulsions.

We have made no experiments with the parotid secretion of other orders of reptiles or other classes of vertebrata, to ascertain whether or no the normal parotid saliva of animals other than snakes has any toxic properties when subcutaneously injected. Such experiments would be a natural continuation of the present inquiry. Especially would it be interesting, in view of its curious snake-like tongue and its persistently evil reputation, not only among the natives of India but also among educated Europeans, to test the saliva of the great "water-lizards" of the genus *Varanus*.

Finally we have to explain that we used the extract of the parotid gland because, even with Opisthoglyphous snakes, we were unable to devise any satisfactory way of getting parotid secretion free from the ordinary saliva of the labial glands.

II. Details of Experiments.

A. *Experiments with Opisthoglyphous Colubrines.*

i. Cerberus rhynchops.

Experiment 1.—A white mouse was injected under the skin of the back with the watery extract of both parotid glands of a full-grown snake. Ten minutes afterwards it was sluggish, 17 minutes afterwards it was lazy and sleepy, and 22 minutes afterwards its hind legs were partially paralysed. In 24 minutes the respirations were at the rate of forty in 15 seconds, and the animal could not stand. In half an hour convulsions began, and the respirations were thirty-seven in 15 seconds. At the 32nd minute the respirations had fallen to fourteen in 15 seconds, and at the 34th minute they had stopped. Thirty-six minutes after the injection the heart stopped also.

Post-mortem.—There was very marked subcutaneous extravasation of blood over the whole of the back.

No control experiments with blood-serum or with the secretion of other glands were made.

ii. Dipsas Forstenii.

Experiment 2.—A black-and-white mouse was injected under the skin of the back with the watery extract (4 minims) of one parotid gland of a full-grown snake. Twelve minutes afterwards spasmodic twitchings began. In another minute the animal turned over on its side, still twitching, and its respirations were thirty-eight in 15 seconds. At the 14th minute it could not stand, and its breathing became slower. At the 16th minute the respirations were seven in 15 seconds, and very laboured. At the 17th minute the heart was beating fast, though the breathing was occasional and convulsive. At the 21st minute after injection the breathing had stopped, and at the 23rd minute the heart.

Experiment 3.—As a control experiment, a white mouse was injected under the skin of the back with the watery extract (4 minims) of one Harderian gland of a *Dipsas Forstenii*. Thirteen minutes afterwards the animal appeared to be slightly sleepy, but no definite symptoms of poisoning were seen within the next hour, and the following day it was quite well.

iii. Dryophis mycterizans.

Experiment 4.—A small black-and-white mouse, whose respirations were about twenty-five in 15 seconds, was injected under the skin of the back with an extract of one parotid gland, in 4 minims of normal salt solution, of a full-grown snake. Two minutes afterwards the animal was restless, 3 minutes afterwards it was restless and twitching, and 5 minutes afterwards it was sleepy and nodding. At the 7th minute it was again restless, and the respirations, which were thirty-eight in 15 seconds, were deep. Twitching continued; but at the 16th minute, though the animal looked sleepy and dejected, it could walk fairly well, and appeared to be improving.

At the 26th minute a similar extract of the other parotid gland of the same snake was injected in the same way. At the 29th minute the animal was again sleepy, and its respirations were thirty-eight in 15 seconds. At the 37th minute it was very sleepy. At the 48th minute it lay quite quiet with its nose on the table, the hind legs appearing to be weak. At the 52nd minute it could not stand, and the respirations were slow and deep. At the 55th minute the respirations were only five in 15 seconds, and of a gasping character, and the animal lay helpless on its side. At the 56th minute convulsions occurred, and respiration then ceased, and just before the 57th minute after the first injection the heart stopped.

Post mortem.—There was most extensive extravasation of blood under the skin of the back and head.

Experiment 5.—A medium-sized black-and-white mouse was injected under the skin of the back with the extract of both parotid glands of a full-grown *Dryophis mycterizans* made by means of 4 minims of a normal saline solution. At the 2nd minute the animal was excited; at the 5th minute it was nodding, its respirations being then thirty-seven in 15 seconds; and at the end of a quarter of an hour it was still sleepy and breathing at the same rate, though deeper than before, with occasional twitching. At the 28th minute it was quiet, but subject to starts, its respirations being thirty-eight in 15 seconds, and of a laboured and occasionally convulsive character. At the 32nd minute the hind legs were weak, and walking was slow and difficult: the respirations were thirty-five in 15 seconds, and laboured. At the 35th minute the hind legs were paralysed and sprawling, and walking was impossible: the respirations were thirty-four in 15 seconds, and very deep. At the 37th minute the animal could just stand with its nose resting on the table: the respirations were thirty-two in 15 seconds, laboured, and occasionally convulsive. At the 39th minute the respirations had fallen to twelve in 15 seconds, and were very laboured: general convulsions then set in, the animal rolled over on its side, and breathing stopped. Forty minutes after the injection the heart stopped also.

Post mortem.—There was very marked extravasation over the back at and beyond the site of injection.

Experiment 6.—As a control experiment, a small black-and-white mouse was injected under the skin of the back with 4 minims of fresh serum of the same *Dryophis* whose glands were used in Experiment 5. The animal was closely watched for 70 minutes, but no symptoms of poisoning appeared, and it was quite well several days afterwards.

Remarks on Experiments 1, 2, 4, *and* 5.

No one who has experimented with minimal lethal doses of Cobra venom can fail to be struck with the close resemblance of the symptoms caused by it with those recorded in the above experiments with the parotid secretion of *Dryophis* and its Opisthoglyphous allies.

The gradual quickening of the respiration, the drowsiness and nodding of the head, with jerky recovery every now and then, followed by gradually increasing paralysis, and a rapid failure of the respirations after they have become laboured in character, by convulsions, and finally by stoppage of the heart some little time after the breathing has ceased, form a sequence of events that, except for a difference in intensity, are common to both, as also is the *post-mortem* picture of subcutaneous extravasation.

B. *Experiments with Aglyphous Colubrines.*

iv. ZAMENIS MUCOSUS.

Experiment 7.—A black-and-white mouse was injected under the skin of the back with half the extract in distilled water of one parotid gland of a large snake. The extract, instead of being a thin opalescent fluid as in the Opisthoglyphous snakes, resembled ropy mucus. Eight minutes afterwards the animal appeared to be quite well, but in 21 minutes from the time of injection it died in violent convulsions.

Experiment 8.—A mouse was injected under the skin of the back with 1 c.c. of the watery extract of one parotid gland of a *Zamenis mucosus* nearly $7\frac{1}{2}$ feet long. Five minutes afterwards it looked anxious and depressed. At 7 minutes the respirations were twenty-nine in 15 seconds. At 9 minutes the hind legs were dragging slightly. At 10 minutes the respirations were twenty-eight in 15 seconds, and the animal could walk fairly well. At 14 minutes the respirations were nineteen in 15 seconds and very laboured: the animal could still walk. At 17 minutes the respirations were ten in 15 seconds, laboured and gasping. At 18 minutes the animal was extremely restless. At 20 minutes violent convulsions and leaping occurred, which ended in the animal rolling over on its side, the

breathing having stopped, though the heart continued to beat faintly. Twenty-two minutes after the injection the heart stopped.

Experiment 9.—A white rat was injected with 1 c.c. of watery extract of one parotid gland of the same *Zamenis* used in Experiment 8. No untoward symptoms occurred.

v. Tropidonotus piscator.

Experiment 10.—A small white mouse was injected under the skin of the back with an extract of both parotid glands of two small specimens of this species, made with 4 minims of normal salt solution. Before the injection the respirations were thirty-six in 15 seconds. Seven minutes afterwards the respirations were thirty-five in 15 seconds and deeper, and at 13 minutes the respirations became laboured and the animal restless. From the 18th to the 23rd minute the respirations became more and more laboured, and increased from thirty-seven to forty in 15 seconds, the animal's restlessness and distress also increasing. At the 32nd minute the respirations, which were deep, had fallen to thirty-three in 15 seconds, the animal half reclining. A minute afterwards the respirations, which were very deep and forced, were twenty in 15 seconds. Convulsions then began, during which the animal rolled over and nearly stopped breathing. At 34 minutes breathing ceased, and just before the 36th minute from the moment of injection the heart stopped.

Post mortem.—There was marked extravasation of blood.

Experiment 11.—A small black-and-white mouse was injected under the skin of the back with the extract in distilled water of one parotid gland of a large *Tropidonotus piscator*. The fluid consisted of thick ropy mucus. Except that its respirations rose rather irregularly from thirty to forty in 15 seconds, and that it scratched itself violently at the site of the injection, the animal seemed for a long time to be all right. At 77 minutes the respirations were thirty-six in 15 seconds, and the animal seemed to be somewhat sleepy. At 92 minutes the animal was distinctly sleepy, moving sluggishly on being stirred, and dragging the hind legs. After this the animal could not be watched, but next morning (15 hours after the injection) it was found dead in its cage.

Experiment 12.—As a control experiment, a white mouse, of the same size as the one used in Experiment 10, was injected under the skin of the back with an extract in 4 minims of normal salt solution of both Harderian glands of both the specimens of *Tropidonotus* used in Experiment 10. The animal was closely watched for 40 minutes, but no ill-effects were observed. It was then put into its cage and looked at from time to time, but it was still quite well and active 6 hours after the injection, and next day its condition was quite normal.

Experiment 13.—As a control experiment another white mouse, of

III. Tabulated Summary of Experiments.

No.	Snake.	Nature of injection.	Animal.	Symptoms began.	Result.	Remarks.
1	*Cerberus rhynchops*....	Watery extract of both parotids	Mouse	In 17 minutes	Died in 36 minutes	Local extravasation.
2	*Dipsas Forstenii*.....	Watery extract of one parotid	,,	In 12 minutes	Died in 23 minutes	,, ,,
3	,, ,, ...	Watery extract of one Harderian gland	,,	No symptoms	—	No effect.
4	*Dryophis mycterizans*..	Normal saline solution, one parotid, repeated in 26 minutes	,,	In 5—7 minutes	Died in 56 minutes	Local extravasation.
5	,, ,, ...	Normal saline solution, both parotids	,,	In 5 minutes	Died in 40 minutes	,, ,,
6	,, ,, ...	4 minims fresh serum.....	,,	No symptoms	—	No effect.
7	*Zamenis mucosus*.....	Watery extract, half of one parotid	,,	?	Died in 21 minutes	Watery effusion.
8	,, ,, ...	Watery extract of one parotid	,,	In 9 minutes	Died in 22 minutes	Violent convulsions.
9	,, ,, ...	,, ,,	Rat	No symptoms	—	
10	*Tropidonotus piscator*	Normal saline solution, both parotids of two individuals	Mouse	In 13 minutes	Died in 35—36 minutes	Local extravasation.
11	,, ,,	Watery extract of one parotid	,,	In 77—92 minutes	Died.	
12	,, ,,	Normal saline solution, both Harderian glands	,,	No symptoms	—	No effect.
13	,, ,,	4 minims fresh serum.....	,,	,,	—	,,

the same size as those used in Experiments 10 and 12, was injected under the skin of the back with 4 minims of fresh serum of one of the specimens of *Tropidonotus* used in those experiments. No effects whatever were produced, though the animal was closely watched for a time and kept under observation until the next day.

Experiments 12 and 13 were subsequently confirmed.

Remarks on Experiments 8, 10, *and* 11.

The parotid extract of the Aglyphous snakes used was a viscid mucus, quite different from the thin opalescent fluid obtained from the Opisthoglyphous snakes. Its chemical nature would also seem to be different, the effects being much less like those produced by minimum doses of Cobra venom.

The violent general convulsions that followed the administration of *Zamenis* extract seem to point to some direct effect upon the nervous system, and are in marked contrast with the dyspnœic convulsions that characterise poisoning by the Opisthoglyphous snakes used in the first series of experiments.

"The Influence of High Pressures of Oxygen on the Circulation of the Blood." By LEONARD HILL, M.B., F.R.S., and J. J. R. MACLEOD, M.B., Mackinnon Research Scholar of the Royal Society. Received May 22,—Read June 12, 1902.

In a former communication* one of us recorded the effect of a pressure of two to three atmospheres on the circulation.

We have since carried on the observations at much higher pressures and by a different method.

A tubular steel pressure chamber was constructed. The ends of the tube were closed by thick glass discs.

A curarised frog was placed inside, and the web of one foot stretched on a wire ring just behind one of the glass discs. The pressure chamber was placed in front of an arc light, and the web illuminated so that the capillary circulation could be observed through a microscope (1-inch objective). The pressure was rapidly raised to 70 atmospheres by connecting the chamber with an oxygen cylinder.

The capillary circulation continued. No alteration could be detected during the rise of pressure. After 15–20 minutes the oxygen tap was closed and the pressure chamber rapidly decompressed. For the first half-minute there occurred no change in the circulation. Then

* 'Roy. Soc. Proc.,' 1900.